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A. Construction of pvvNADLd1ubiNS2

1. RT-PCR fragment 1
Bovine ubiquitin

2. PCR fragment 2
NS3 to Nsi I

3. Restrict PCR fragment 3
with PinA I and Nsi I

4. Ligate fragment 3
with pvvNADLd1NS2
restricted with PinA I
and Nsi I

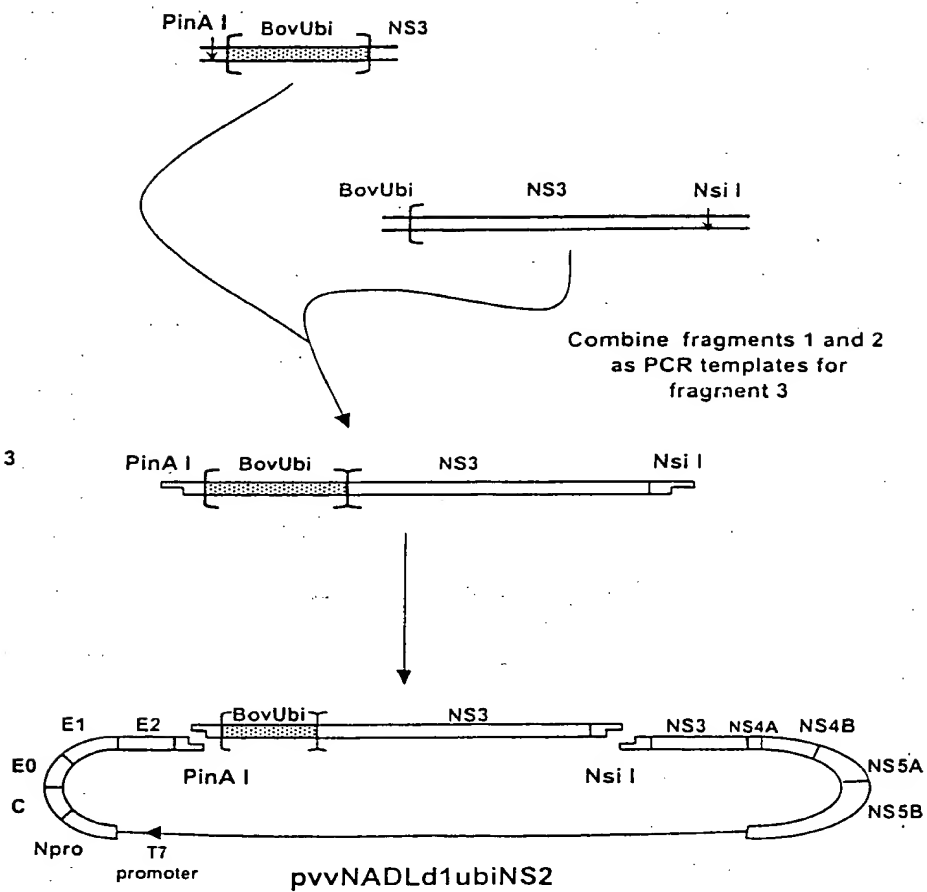
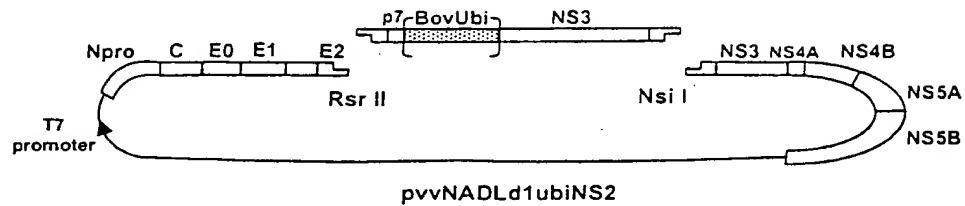


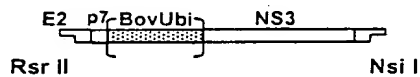
Figure 1A

B. Construction of p15aD1ubiNS2

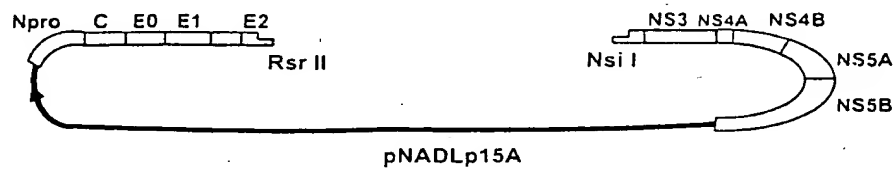
1. Restrict pvvNADLd1ubiNS2 with Rsr II and Nsi I



2. Isolate Rsr II and Nsi I fragment from pvvNADLd1ubiNS2



3. Ligate fragment with pNADLp15A restricted with Rsr II and Nsi I



4. Plasmid p15aD1ubiNS2

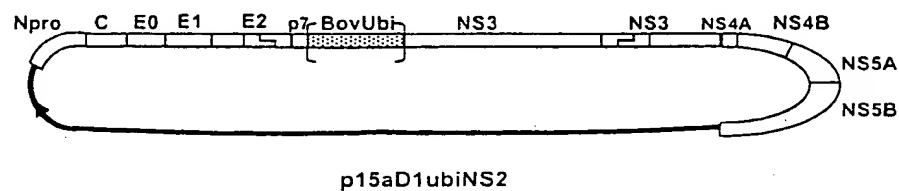
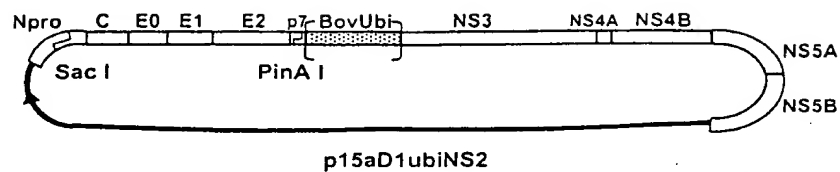


Figure 1B



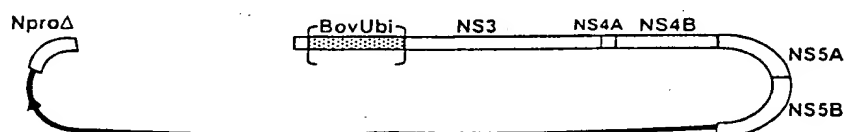
C. Construction of p15aDI

1. Restrict plasmid p15aD1ubiNS2 with Sac I and PinA I



Discard insert

2. Create blunt ends



3. Religate to create p15aDI

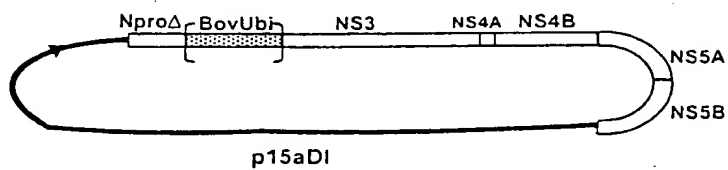
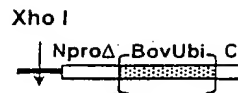


Figure 1C

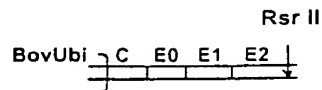


D. Construction of pBVDdN6

1. PCR fragment 1
NproΔ-BovUbi

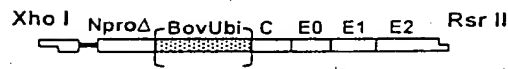


2. PCR fragment 2
BovUbi to E2



Combine fragments 1 and 2
as PCR templates for
fragment 3

3. Restrict PCR fragment 3
with Xho I and Rsr II



4. Ligate fragment 3
with pNADLp15a
restricted with Xho I
and Rsr II

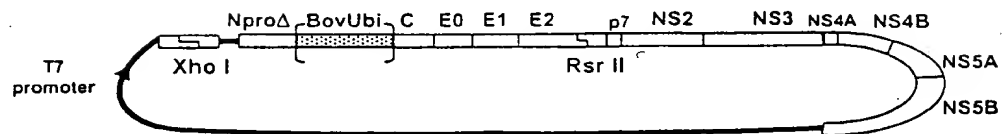


Figure 1D

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FIGURE 2A



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FIGURE 2A

JAN 07 2004

TRADEMARK OFFICE

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SEQ ID NO:11

FIGURE 2A

SHEET 3 OF 3



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FIGURE 2B



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FIGURE 2B

[illegible]

FIGURE 2B



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SEQ ID NO:12

FIGURE 2B

SHEET 4 OF 4

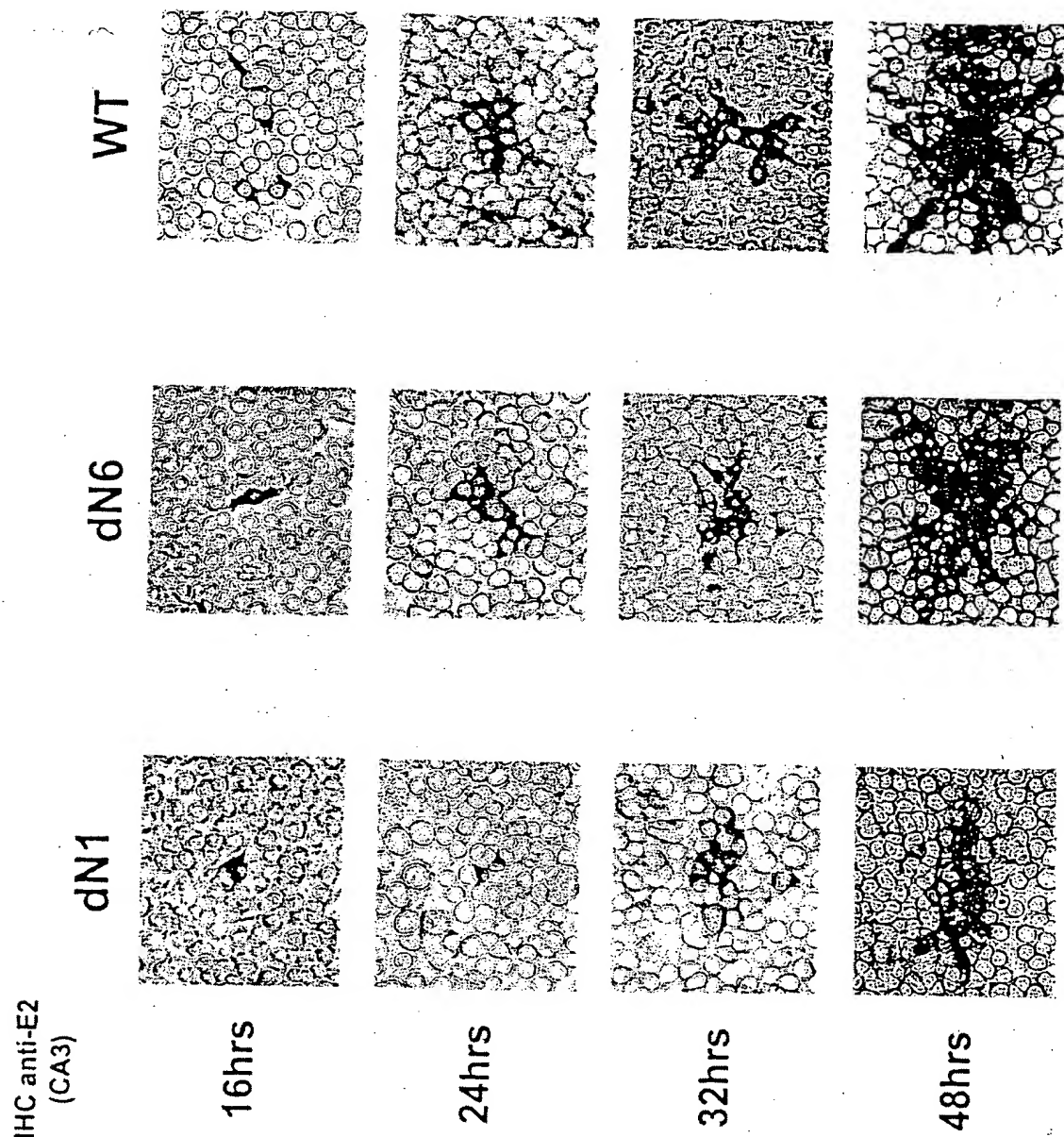


FIGURE 3



GROWTH CURVE OF BVDV MUTANTS IN MDBK CELL SYSTEM

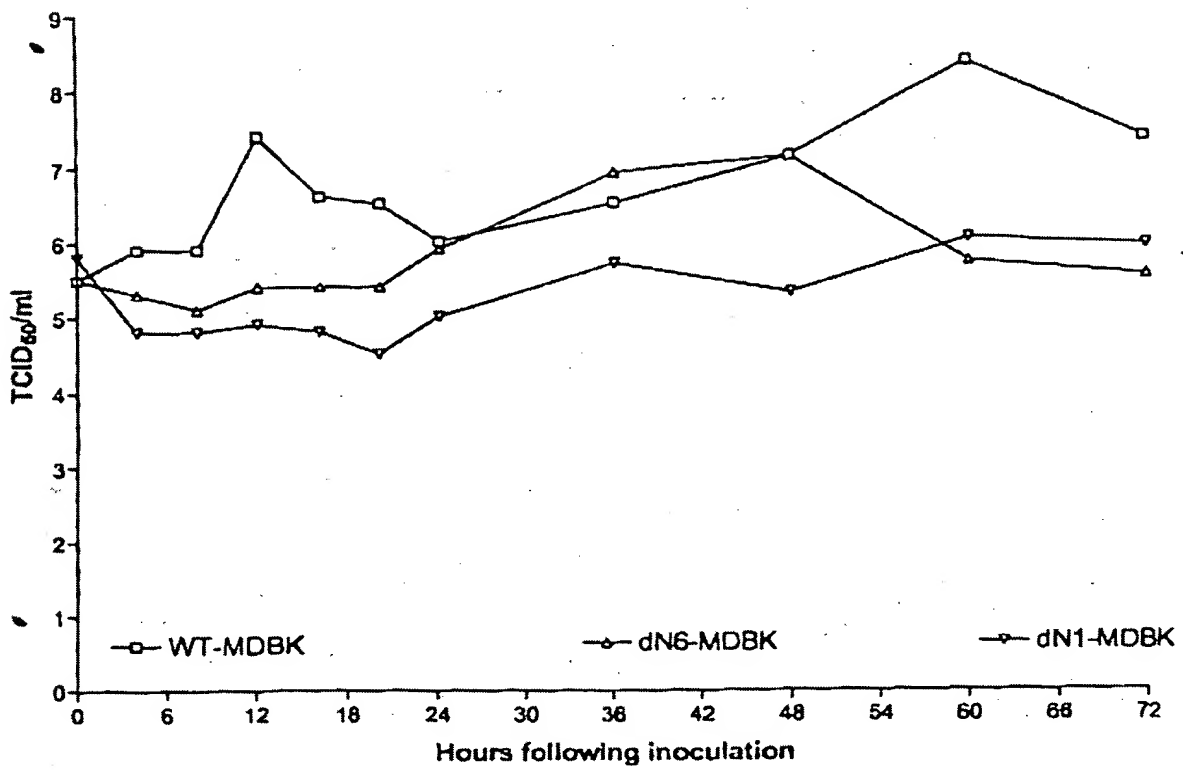


FIGURE 4